Award Number:
W81XWH-07-1-0244

TITLE:
The Root Cause of Post-traumatic and Developmental Stress Disorder

PRINCIPAL INVESTIGATOR:
Keith A. Young, PhD

CONTRACTING ORGANIZATION:
Texas A&M Health Science Center
Temple Texas 76504

REPORT DATE: March 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:)

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
March 2013	Annual	1 March 2012- 28 Feb 2013

4. TITLE AND SUBTITLE
The Root Cause of Post-traumatic and Developmental Stress Disorder

5a. CONTRACT NUMBER
W81XWH-07-1-0244

5b. GRANT NUMBER
W81XWH-07-1-0244

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Keith A. Young, PhD

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Texas A&M Health Science

Temple Texas 76504

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
MOMRP

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our overarching scientific hypothesis holds that serotonergic influences on brain development driven by genetics and early experience induce a variation of normal brain anatomy that makes the brain highly susceptible to the effects of severe stress. We are studying this question using both clinical and basic approaches. New findings from our lab funded by VA support the existence of an anatomical phenotype conferring susceptibility to depression, and the current work seeks to extend these findings to PTSD. After TATRC review in January of 2011, a revised research plan was developed to include a pre/post-deployment study at Fort Hood and anatomical studies of PTSD in collaboration with NIMH, Yale and USUHS. Based on input from contracting relating to the maturation date of funds, the budget and revised proposal was resubmitted in December and the funds were released for use in June, 2012. Eight new Fort Hood staff were hired and certified to perform the SCID and Columbia suicide interviews. Post-mortem brain tissue from 30 PTSD, 30 MDD and 30 controls are being used to investigate genomics and epigenetics in several areas of the cortex and thalamus. Golgi methods for analysis of prefrontal anatomy and stereological studies of the frontal cortex in Nissl sections are in progress. Work on both clinical and preclinical studies continues uninterrupted into the second phase of funding.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**		9	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

INTRODUCTION:

This research has been funded in two installments (Phase I and Phase II contracts). The research described below is continuing without interruption through 2016 with implementation of the phase II contract, when the main body of the data will be available for analysis and publication. Our overarching scientific hypothesis holds that serotonergic influences on brain development driven by genetics and early experience induce a variation of normal brain anatomy that makes the brain highly susceptible to the effects of severe stress. The goal of Project 1 is to describe the progression of post-deployment stress disorders (PTSD, major depression, suicidality) in active duty troops using predeployment/postdeployment structured clinical interviews, and to investigate developmental and environmental factors that influence predisposition to PTSD and depression. A subset of participants will be selected to have predeployment/postdeployment MRI and psychophysiological analysis. Using DNA gathered from clinical trials, we will investigate genetic factors influencing resiliency and susceptibility to stress disorders using a panel of 20 genes that we have tested and validated. Project 2 will investigate post-mortem anatomy in subjects with major depression and/or PTSD. Both molecular and histological techniques will be employed to study the brains already collected. An overarching goal of the Program is integration of data across the projects to compare and contrast the potential for different assessment paradigms (MRI anatomy, fMRI, evoked potentials, startle, genetic profiling) to screen for resiliency and predisposition to post-traumatic and developmental stress disorder stress disorders.

BODY:

KEY RESEARCH ACCOMPLISHMENTS:

Administrative:

Approval to move forward with the redesigned Project 1 was received from TATRC and MOMRP in February, 2012 and the redesigned budget was released in June, 2012. The IRB for Project 1 has completed initial review at BAMC and is under review by HRPO. Approval for the post-mortem human work was received from ORP in September, 2012. The transition from the Phase 1 to Phase II funding to support the research was accomplished in May, 2013 and we are now running on the Phase II funding through 2016.

Project Specific:

Project 1: Longitudinal study

Task 1: Sample 2000 active duty/guard troops predeployment

a. Diagnostic interview (SCID)
b. Depression symptoms
c. Stress battery (DRRI, development history, suicidality)
d. Blood for DNA/RNA
e. Medical testing (CBC/TSH/CMP)

Task 2: Resample/test post-deployment

Initial IRB review is completed at BAMC and the proposal has been submitted to HRPO for initial review. Seven SCID interviewers completed training and were certified by our training team. Columbia Suicide Interview training and phlebotomy training is complete and all staff are certified. The team is established and centrally located on the Fort Hood Cantonment embedded in the Resiliency Training Program Facility in Building 20112, part of the Resiliency Campus. Contact with units set to deploy in the next 9 months have been established and they are supportive of soldier participation.

Project 2 Neurobiology

Task 1: Pre-deployment/post-deployment MRI testing *300 scanning sessions*

IRB approval for MRI work is under review at BAMC and we are working through the 1st set of stipulations.

Task 2. Collection of PTSD, MDD and control brains

A total of 30 PTSD, 30 MDD (psychiatric controls) and 30 normal controls have been diagnosed and comprise our current cohort, by far the largest PTSD post-mortem cohort in existence. Initial studies on this tissue is underway as described below. Additional specimens continue to be accrued into the collection, which is being

Task 3. Compare gene expression in the frontal cortex of PTSD and controls.

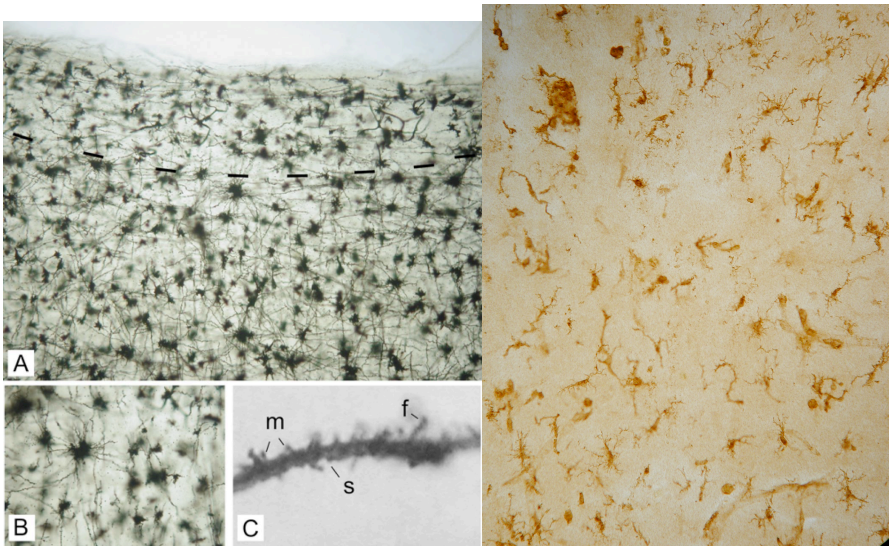
Sample set 1) Frontal cortical tissue (area 9/25) from 23 PTSD (Appendix), 25 MDD and 25 controls have completed gene expression and methylation procedures. RNAseq analysis will be performed on these samples starting in the next 6 months. Data cleaning and analysis are being performed at this time and we expect initial publications within 9 months.

Sample set 2) In the medial orbitofrontal cortex in a subset of these brains (N=8 PTSD and 8 controls), we have documented hypermethylation of transcription start sites on 355 genes in PTSD brains (Table 2), compared to 11 PTSD hypomethylated regions. The overabundance of hypermethylation suggests abnormal pathophysiological processes such as altered metabolism, impairments in neuronal or glial physiology, or changes in the ratios of cells such as would occur with microgliosis. We are in the process of extending this

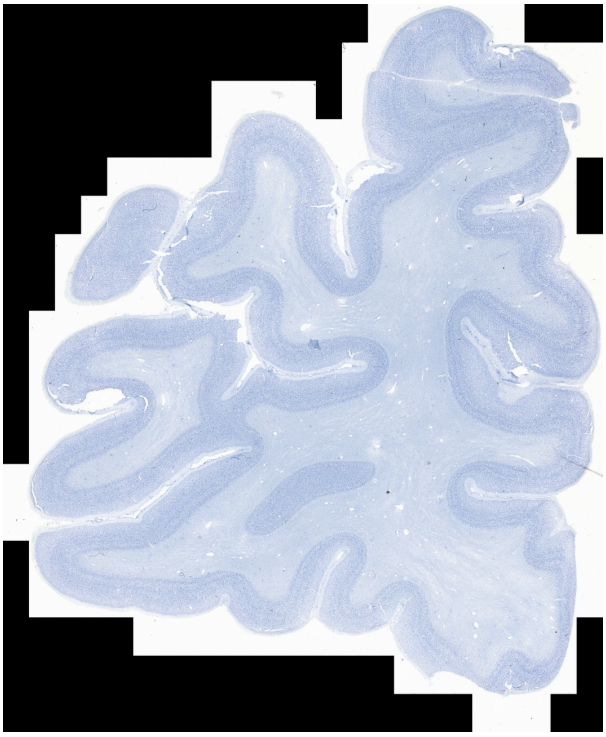
data to adding microRNA and gene expression, and have obtained tissue from the larger cohort to follow up on this finding with a larger N (N=30 PTSD, 30 MDD and 30 Controls).

Task 4. Compare anatomical markers in frontal cortex/hippocampus of PTSD, MDD and controls, with 5HTTLPR and other genetic variants as cofactors.

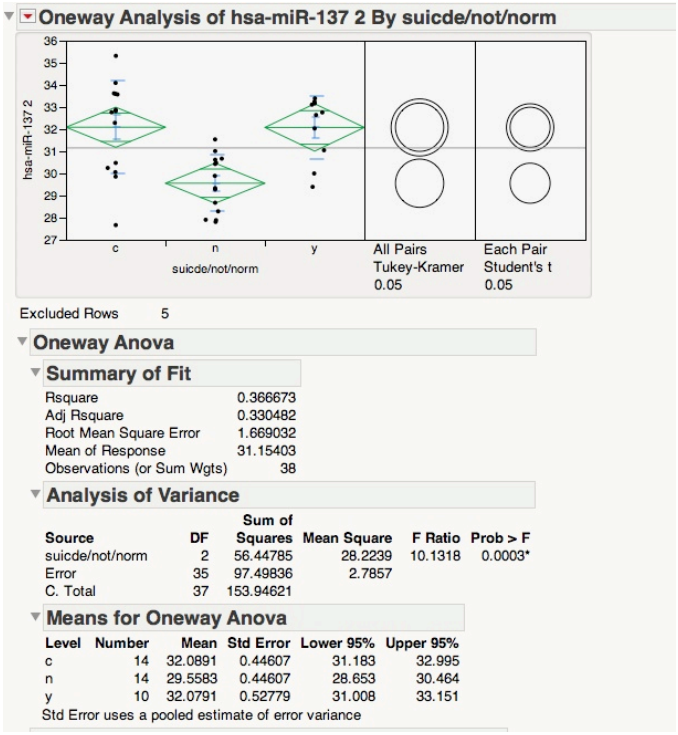
A. Golgi staining (left) and microglia staining (right) in the mOFCTX has been performed on 8 PTSD and 8 Control specimens. Morphological analysis (starting with dendritic arborization morphology) of the sections is proceeding in Phase II. Additional mOFCT, posterior cingulate and motor cortex (control ROI) from PTSD, MDD and Control tissue is being sampled and will be available for future anatomical studies.



B. Nissl staining on whole hemisphere sections from the frontal cortex of 8 PTSD vs 8 controls is being completed in phase II of the study. An initial neuron and glial cell counting study on the straight gyrus of the mOFCTX is approximately 30% completed. This data will be compared and contrasted with gene expression and methylation data from the same region of the opposite hemisphere of these brains. We will also compare the stereology results with spine counts and arborization studies from this area.



C. MicroRNA (miR) levels from frozen mediodorsal thalamus have been analyzed to begin the study of suicide on cellular pathophysiology. As previously reported in the frontal cortex (Smalheiser et al, 2012, MicroRNA Expression Is Down-Regulated and Reorganized in Prefrontal Cortex of Depressed Suicide Subjects. PLoS ONE 7: e33201. doi:10.1371 /journal.pone.0033201), we observed that levels of multiple mRNA's were reduced in a coordinated manner in tissue from individuals who died by suicide. The pattern of miR reductions in suicide brains was present in approximately 1/3 of all mRNAs tested. Interestingly, there was also evidence for elevated miR levels in many of the affected miR's, so those that committed suicide may have experienced a "normalization" of miR levels before suicide. In the below graph, c = normal control, n = psychiatric not suicide, and y = psychiatric suicide. The data below represent housekeeping gene corrected amplification, so larger numbers = reduced levels. This data highlights that care is needed obtain good data on suicidality in mood disorder post-mortem studies. We are using this data to generate and test a more efficient mRNA platform that uses a subset of mRNAs to capture most of variable effects with a greatly reduced cost. We expect a publication this year from this data.



REPORTABLE OUTCOMES: None
CONCLUSION: No scientific conclusions have been made at this point in time.

APPENDICES:
Appendix 1: PTSD specimen characteristics
Appendix 2: Top 20 hypermethylated transcription start sites in PTSD OFC

Appendix 1: PTSD specimen characteristics:

ID	Age	Gender	Race	pH	PMI	Manner	Smoke	DX	RIN
1924	41	M	AA	6.78	28	Accident	Yes	PTSD	8.3
1938	27	M	CAUC	6.74	19	Accident	Yes	PTSD/MDD	8.7
2058	40	M	CAUC	6.64	29.5	Accident	Yes	PTSD/MDD	8.7
2324	27	M	CAUC	6.38	25	Accident	Yes	PTSD/BP	5
2498	52	M	CAUC	6.9	22	Homici	Yes	PTSD/MDD	9.3

2499	55	M	HISP	6.1	14	Accident	Yes	PTSD/MDD	3.9
2500	59	M	HISP	6.7	24	Natural	Yes	PTSD/MDD	8.6
2502	52	M	HISP	6.4	12	Natural	No	PTSD/MDD	9
2503	41	M	CAUC	6.7	36	Natural	Yes	PTSD	9
2504	61	M	CAUC	6.1	16.5	Natural	Yes	PTSD	9.5
2505	39	F	AA	6.2	23.5	Accident	No	PTSD/MDD	8.6
1513	58	F	CAUC	6.01	28.5	Natural	Yes	PTSD	8.3
2364	48	M	CAUC		21	Accident	Yes	PTSD/BP	IP
2379	20	F	CAUC	6.5	13	Suicide	Yes	PTSD	IP
2387	31	M	CAUC	6.8	28.5	Accident	Yes	PTSD	8.6
2404	57	F	CAUC	6.53	34	Suicide	Yes	PTSD/MDD	IP
2427	23	M	CAUC	6.58	27.5	Accident	Yes	PTSD/BP	IP
2443	24	M	CAUC		23	Suicide	U	PTSD	IP
2444	26	M	AA		30.5	Natural	Yes	PTSD	IP
2466	43	F	CAUC			Natural	Yes	PTSD	IP
2507	34	F	AA		32	Natural	Yes	PTSD	IP
2515	30	F	CAUC		16.5	Suicide	No	PTSD	IP
2523	41	F	CAUC	6.58	36	Natural	U	PTSD/MDD	7.2

IP = in process

Appendix 2: Top 20 hypermethylated transcription start sites in PTSD OFC

C	region start	– end	type	P	GeneID	fold	Gene	
1	43001342	43009342	core	0.00001	64175	-2.3	LEPRE1	LEUCINE- AND PROLINE-ENRICHED PROTEOGLYCAN 1
17	7679959	7687959	core	0.00001	23135	-2.18	KDM6B	LYSINE-SPECIFIC DEMETHYLASE 6B
7	44751054	44759054	core	0.00001	83637	-1.62	ZMIZ2	ZING FINGER MIZ-DOMAIN CONTAINING 2
1	39925676	39933676	core	0.00001	51440	-1.54	HPCAL4	Hippocalcin like 4,
12	65945327	65953327	core	0.00001	55832	-1.47	CAND1	CULLIN-ASSOCIATED NEDDYLATION-DISSOCIATED PROTEIN 1
19	3932461	3940461	core	0.00001	1938	-1.47	EEF2	EUKARYOTIC TRANSLATION ELONGATION FACTOR 2
7	32893993	32901993	core	0.00001	25948	-1.4	KBTBD2	kelch repeat and BTB (POZ) domain containing 2
18	2557489	2565489	core	0.00001	64863	-1.31	METTL4	METHYLTRANSFERASE-LIKE 3
12	52630980	52638980	shore	0.00001	3228	-1.29	HOXC12	HOMEOBOX C12
7	154939584	154947584	shore	0.00001	2020	-1.28	EN2	ENGRAILED 2
3	5200358	5208358	core	0.00001	9695	-1.27	EDEM1	ER DEGRADATION-ENHANCING ALPHA-MANNOSIDASE-LIKE PROTEIN 1
15	76513572	76521572	core	0.00001	3658	-1.25	IREB2	IRON-RESPONSIVE ELEMENT-BINDING PROTEIN 2
14	31611656	31619656	core	0.00001	84837	-1.22	C14orf128	ARHGAP5-AS1
22	22699116	22707116	shore	0.00001	391322	-1.22	LOC391322	D-dopachrome tautomerase-like
8	25094203	25102203	core	0.00001	80005	-1.1	DOCK5	Dedicator of cytokinesis 5
19	40179085	40187085	core	0.0001	57655	-2.01	GRAMD1A	GRAM domain containing 1A
4	14610619	14618619	core	0.0001	132864	-1.98	CPEB2	CYTOPLASMIC POLYADENYLATION ELEMENT-BINDING PROTEIN 2
12	26165270	26173270	core	0.0001	79365	-1.92	BHLHE41	BASIC HELIX-LOOP-HELIX FAMILY, MEMBER E41
20	52521672	52529672	core	0.0001	55816	-1.85	DOK5	DOCKING PROTEIN 5

Methyl binding domain capture (MBDCap)was used to isolate and determine methylation levels for transcription start sites (8000 bases upstream) in DNA extracted from the medial orbitofrontal cortex (mOFCtx) and posterior cingulate cortex (pCCtx) in PTSD (N=7) and controls (N=7). Uncorrected P values for the differentials found 355 hypermethylated regions with $P<0.01$, but only 11 sites were hypomethylated at a $P<0.01$ level in PTSD specimens. Two of the hypermethylated top 20 sites (EEF2 and CPEB2) are interacting proteins that are involved in the translation of BDNF and other proteins in neuronal processes, particularly spines. This pattern of preferential hypermethylation in PTSD is not present in the pCCtx in the same subjects, suggesting that a selective pathophysiological process is occurring in the OFCtx in PTSD. We are analyzing 20+ additional PTSD and control specimens to validate this finding, and are including 30 MDD subjects so that we can determine if the changes are specific to PTSD or are shared by other stress-related disorders.